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Proventriculitis in Broiler Chickens: Effects of Immunosuppression

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SUMMARY. Proventriculitis in broilers causes carcass condemnation when swollen proventriculi tear during evisceration. The cause of this proventriculitis is unknown, but several infectious agents have been associated with it. One such agent, infectious bursal disease virus (IBDV), has been implicated as a cause of proventriculitis, but a direct effect of this virus on the proventriculus has not been proven. The role of IBDV in proventriculitis may be indirect as a result of its ability to cause immunosuppression. The objective of this study was to understand how immunosuppression affects the incidence of proventriculitis in broiler chickens. Immunosuppression was induced in commercial and specific-pathogen-free broiler chickens using chemicals (cyclophosphamide and cyclosporin) or virus (IBDV). All groups were then exposed to a proventricular homogenate produced from diseased birds. At 7 and 14 days postinoculation, the incidence of proventriculitis in these groups was compared to that produced by homogenate exposure in immunocompetent broilers. All birds exposed to the proventricular homogenate from diseased birds developed proventriculitis. Cyclophosphamide and IBDV, both B cell suppressors, did not significantly affect the incidence or characteristics of the proventriculitis observed, although they did have an effect on the size of the proventriculus at 7 days postinoculation. Chickens immunosuppressed with cyclosporin, a T cell suppressor, developed more severe lesions and had a higher incidence of proventriculitis. These findings indicate that both B and T cells are involved in the immune response against proventriculitis, but cell-mediated immunity appears to have a more important role in controlling the disease. IBDV affects both humoral and cellular immunity in the chicken, so although under experimental conditions it didn't have a major effect on proventriculitis, it may explain why control of IBDV in the field seems to reduce the incidence of proventriculitis.

RESUMEN. Proventriculitis en pollos de engorde: efectos de la inmunosupresión.

La proventriculitis en pollos de engorde es causa de decomisos de canales en las plantas de procesamiento, debido a la ruptura de los proventrículos inflamados al momento de la evisceración. La causa de esta proventriculitis es desconocida, pero la presencia de varios agentes infecciosos ha sido relacionada con el proceso. Uno de estos agentes, el virus de Gumboro, ha sido implicado en procesos de proventriculitis, pero los efectos directos del virus sobre el proventrículo no han sido comprobados. El efecto del virus de Gumboro en la proventriculitis puede ser indirecto, siendo el resultado de la capacidad del mismo para inducir inmunosupresión. El objetivo de este estudio fue estudiar el efecto de la inmunosupresión sobre la incidencia de proventriculitis en pollos de engorde. Se produjo inmunosupresión en pollos de engorde comerciales y pollos libres de patógenos específicos (SPF) mediante el uso de químicos (ciclofosfamida y ciclosporina), o mediante desafío viral (virus de Gumboro). Estos grupos fueron luego expuestos a tejido proventricular homogenizado procedente de aves enfermas. Se comparó la incidencia de proventriculitis en estos grupos y en grupos de aves inmunocompetentes inoculadas con el homogenizado de proventrículo, a los 7 y 14 días después del desafío. Todas las aves inoculadas con el homogenizado de proventrículo presentaron síntomas de proventriculitis. La inoculación con ciclofosfamida o el virus de Gumboro, ambos supresores de los linfocitos B, no afectó en forma significativa la incidencia o características de las lesiones de proventriculitis observadas, aunque sí influyó en el tamaño de los proventrículos a los

7 días después del desafío. Los pollos inoculados con ciclosporina, un supresor de los linfocitos T, desarrollaron lesiones más severas y presentaron una mayor incidencia de proventriculitis. Estos hallazgos indican que los linfocitos B y T están involucrados en la respuesta inmune en procesos de proventriculitis, aunque la respuesta inmune de tipo celular parece tener un papel más importante en el control de la enfermedad. El virus de Gumboro afecta las respuestas inmunes de tipo humoral y celular en los pollos, por lo tanto, aunque bajo condiciones experimentales no produjo un efecto marcado en el proceso de proventriculitis, este hecho puede explicar la razón por la cual el control de la enfermedad de Gumboro en el campo parece reducir la incidencia de proventriculitis.

Key words: proventriculitis, immunosuppression, IBDV

Abbreviations: CAV = chicken anemia virus; CBH = cutaneous basophil hypersensitivity; CMI = cell-mediated immunity; CP = cyclophosphamide; CS = cyclosporin; HE = hematoxylin and eosin; IBDV = infectious bursal disease virus; IBV = infectious bronchitis virus; NDV = Newcastle disease virus; PBS = phosphate-buffered saline; PSS = physiological saline solution; RT-PCR = reverse transcriptase–polymerase chain reaction; SPF = specific-pathogen-free

Proventriculitis is a clinical condition that affects broiler chickens. It is characterized by enlargement of the proventriculus and weakness of the gastric isthmus. During routine evisceration at processing, affected proventriculi rupture, causing spillage of the proventricular contents into the body cavity, which results in condemnation of affected carcasses for contamination. The disease has also been associated with impaired growth and poor feed conversion (13,16). Microscopically, degeneration and necrosis of proventricular glands is observed, accompanied by marked intraglandular interstitial lymphocytic infiltration (3,9,10).

Several agents have been implicated as potential causes of proventricular lesions. Noninfectious causes include oral exposure to biogenic amines (2,27), mycotoxins (26), lack of dietary fiber (29), and excessive copper sulfate (4,14,40). Infectious causes include adenovirus (19), reovirus (17,38), infectious bronchitis virus (41), and megabacterium (23,32). However, none of these noninfectious or infectious agents has been found consistently in a majority of cases. Electron microscopy has detected viral particles in acute lesions, but isolation of a virus from affected proventriculi has been unsuccessful (9,10,13).

Infectious bursal disease virus (IBDV) has been implicated as the cause for proventriculitis (3,13,24), and IBDV vaccination has been reported to decrease its incidence (7,15). Proventriculitis can be reproduced by orally inoculating broilers with homogenized proventriculi collected from affected birds (3,16). A filterable agent found in these homogenates causes lesions similar to those found in field cases (3), and IBDV has been immunoprecipitated from these homogenates (13). Commercial broilers exposed to this IBDV developed increased proventricular lesion scores but had no increase in proventricular size, a characteristic feature produced by exposure to proventricular homogenates (13). These findings indicate that other agents or conditions may be required to produce proventriculitis.

IBDV induces immunosuppression in chickens (21,35,39). Immunosuppressed flocks may have an increased incidence of secondary infections, poor feed conversion, and reduced protective response to commonly used vaccines (35). IBDV causes lytic destruction of IgM+ B lymphocytes that results in suboptimal levels of antibodies against a number of infectious and noninfectious antigens (8,30,35). Although the immunosuppression caused by IBDV is principally due to B lymphocyte damage, an effect on cell-mediated immunity (CMI) has also been demonstrated (5,18,33,35).

Specific-pathogen-free (SPF) broilers exposed to different strains of IBDV did not develop proventricular lesions or enlargement at 4 or 6 days postinoculation (25). The virus was detected in large quantities in the bursa of these birds by reverse transcription–polymerase chain reaction (RT-PCR) and immunohistochemical techniques, but not in the proventriculus, indicating it is not a target organ for IBDV. However, the immunosuppressive effect of IBDV could explain its reported relationship to proventriculitis. The purpose of our study was to see if immunosuppression had any effect on the incidence, severity, or character of proventriculitis in broiler chickens. To address this, commercial and SPF 1-day-old broilers were immunosuppressed with cyclophosphamide (B cell suppressor), cyclosporin (T cell suppressor), or IBDV. Subsequently these chickens were exposed to a proventricular homogenate from affected chickens, and the effect of immunosuppression on proventriculitis was determined.

MATERIALS AND METHODS

Animal housing. One-day-old chickens were divided into groups and housed in positive-pressure Horsfal units. Unmedicated feed and water were provided *ad libitum.*

Experimental design. *Trials 1 and 2.* A total of 88 unvaccinated commercial broiler chicks, obtained from a local hatchery, were divided into nine groups of 8 or 12 birds, and chicks in each group received either an immunosuppressive treatment or no treatment (Table 1). Chickens subsequently received, as described below, either positive (+PV) or negative (–PV) proventricular homogenate, saline, or no homogenate. Group 1 had 12 birds, which were inoculated *per os* with 1 ml of sterile saline at 7 days of age. Group 2 had eight birds, which were inoculated *per os* with 1 ml of –PV produced from normal commercial broilers at 7 days of age. Group 3 had eight birds, which were inoculated at 7 days of age *per os* with 1 ml of +PV produced from broilers that had proventriculitis. Group 4 had 12 birds, which were immunosuppressed with IBDV administered at 1 day of age. Group 5 had 12 birds, which were immunosuppressed with cyclophosphamide (CP) starting at 1 day of age. Group 6 had 12 birds, which were immunosuppressed with cyclosporin (CS) starting at 1 day of age. Group 7 had eight birds, which were immunosuppressed with IBDV administered at 1 day of age and treated with +PV at 7 days of age. Group 8 had eight birds, which were immunosuppressed with CP starting at 1 day of age and treated with +PV at 7 days of age. Group 9 had eight birds, which were immunosuppressed with CS starting at 1 day of age and treated with +PV at 7 days of age.

Trial 3. This trial was conducted in a manner similar to that described for trials 1 and 2, with the following modifications. Fertile White Plymouth Rock chicken eggs (SEPRL, USDA, Athens, GA) from a breeder flock maintained under SPF conditions were obtained, hatched, and maintained in positive-pressure Horsfal isolation units. The parent flock and all progeny used in this experiment were free of common poultry diseases, specifically IBDV, Newcastle disease virus (NDV), infectious bronchitis virus (IBV), reovirus, and chicken anemia virus (CAV). The same experimental design and protocol used in trials 1 and 2 was followed. Additional animals were included to allow for a third euthanatization at 21 days postinoculation.

Immunosuppressive treatment groups. Chickens were immunosuppressed with either IBDV, CP, or CS, as described below.

IBDV treatment. Birds in trial 1 (groups 4 and 7) were challenged with IBDV Variant E strain (Intervet, Inc., Millsboro, DE). In trials 2 and 3, chickens in groups 4 and 7 were treated with the STC challenge strain 124-ADV of IBDV (National Veterinary Services Laboratory, Ames, IA). Inoculations were given *per os* and by eye drop and consisted of 100 µl containing at least 10^3 mean tissue culture infective dose of virus diluted in phosphate-buffered saline (PBS).

Table 1. Experimental protocol for trials 1 and 2 (commercial broilers) and trial 3 (SPF broilers). Four birds were necropsied per group on day 14 (7 days postinoculation) and 21(14 days postinoculation) in all three trials and also on day 28 (21 days postinoculation) in trial 3.

Groups	1 day of age immunosuppression treatment[A]	7 days of age homogenate treatment[B]
1. Saline	—	Saline
2. –PV	—	–PV
3. +PV	—	+PV
4. IBDV	IBDV	—
5. CP	CP	—
6. CS	CS	—
7. IBDV/+PV	IBDV	+PV
8. CP/+PV	CP	+PV
9. CS/+PV	CS	+PV

[A]IBDV treatment: 10^3 CID_{50} *per os* strains Variant E (trial 1) or STC (trials 2 and 3). Cyclophosphamide (CP) treatment: 4 mg intraperitoneally for 4 days starting at 1 day of age. Cyclosporin (CS) treatment: intramuscular injection of 50 mg/kg body weight every third day, starting at 1 day of age.

[B]Saline: 1 ml sterile saline *per os*; –PV = proventricular homogenate from normal chickens, 1 ml *per os*; +PV = proventricular homogenate from chickens with proventriculitis, 1 ml *per os.*

CP treatment. B lymphocyte immunosuppression was induced using an established protocol (34). Briefly, groups 5 and 8 in all three trials received one intraperitoneal injection of 4 mg CP (cyclophosphamide monohydrate, 0.1 ml; Sigma Chemical Co., St. Louis, MO) daily for 4 days starting the first day after hatch. For injection, CP was obtained in a dry form, and an aqueous solution was prepared by reconstituting 1.6 g in 40 ml of calcium- and magnesium-free phosphate-buffered sterile saline and filtering this solution through an 0.22-µm syringe filter. The resulting solution contained 40 mg of CP/ml.

CS treatment. T lymphocyte immunosuppression was induced using an established protocol (31). Briefly, chickens from groups 6 and 9 in all three trials received one intramuscular injection of CS, 100 mg/kg body weight, every 3 days from the first day after hatch until the experiment ended. CS was prepared by diluting a stock solution (Sandimmune, 100 mg/ml, Novartis Pharma AG, Basle, Switzerland) 1:1 in olive oil. Dilutions of the drug were adjusted as body weights increased.

Immunosuppression in IBDV-, CP-, and CS-treated

groups was assessed by histopathologic examination of immune organs (bursa, thymus, and spleen) cutaneous basophil hypersensitivity (CBH) response testing, and humoral response to NDV vaccination.

Challenge with proventricular homogenates. At 7 days of age, birds from groups 3, 7, 8, and 9 in trial 1 were inoculated by oral gavage with 1 ml of a positive proventricular homogenate (+PV). This homogenate was prepared using affected proventriculi obtained from a flock of 4-wk-old Cornish hens with proventriculitis in whom proventriculi were processed and homogenized as described (3). Proventriculi from chickens in group 3 (+PV treated) of trial 1 were also homogenized as previously described (3) and used to expose +PV groups in trials 2 and 3. Briefly, proventriculi collected from birds that developed proventriculitis were washed in sterile normal saline (PBS) three times on a magnetic stirrer to remove feed residues and toxins. Washed proventriculi were then diluted 1:1 w/v in PBS and homogenized with a commercial blender (Waring, New Hartford, CT). The homogenates were frozen at −80 C and thawed at room temperature immediately before inoculation. The same procedure was followed using proventriculi from normal unvaccinated 14-day-old commercial broiler chickens obtained at 1 day of age from a local hatchery and raised in isolators. This negative proventricular homogenate (−PV) was used to inoculate birds from group 2 in all three trials. +PV and −PV were not tested for the presence of viruses, although IBDV was isolated from the original +PV (13). Birds of group 1 in all trials were sham inoculated with 1 ml of sterile saline.

CBH response. This test was performed to evaluate T cell function in the immunosuppression treatment groups at 2 wk of age, as previously described (6). Four chickens from control groups 1 (saline), 4 (IBDV), 5 (CP), and 6 (CS) were injected intradermally in the skin between the third and fourth digits of the left foot with 200 μg of Phytohemmagglutinin-P (PHA-P, Sigma Chemical Co.) in 100 μl of sterile physiological saline solution (PSS). The right foot of each chicken was similarly injected with 100 μl of PSS without PHA-P to serve as a control. The CBH response to PHA-P was evaluated by determining the thickness of the interdigital skin before injection and at 12 and 24 hr after injection with a constant-tension, digital micrometer (Mitotuyo Co., Kanagawa, Japan). The CBH response was calculated using two methods: (1) CBH-1 or increased skin thickness = (postinjection skin thickness, left foot) − (preinjection skin thickness, left foot); and (2) CBH-2 response = (PHA-P response, left foot) − (PSS response, right foot).

NDV vaccination. To assess humoral immune function, four birds from control groups 1 (saline), 4 (IBDV), 5 (CP), and 6 (CS) were vaccinated at 21 days of age with killed Newcastle disease vaccine (Vineland Laboratories, Vineland, NJ). Each bird was given one dose of 0.5 ml of vaccine intramuscularly, as recommended by the manufacturer. Two weeks later, birds were bled to obtain sera, and antibodies to NDV were quantified by enzyme-linked immunosorbent assay (ELISA) (IDEXX Laboratories, Inc., Westbrook, ME), and hemagglunation inhibition test using the diluted serum–constant virus procedure (37).

Sample collection and processing. All birds were wing banded and weighed at 1 day of age. At 14 and 21 days of age, four birds were randomly selected from each group and examined, weighed, bled, euthanatized by cervical dislocation, and necropsied. Bursa, proventriculus, spleen, and thymus were collected from each bird, weighed, and a portion of each was fixed immediately by immersion in 10% neutral buffered formalin for 24 hr. Tissues were then processed and embedded in paraffin using routine histologic techniques. The remaining proventriculi were collected in sterile plastic tubes over ice for subsequent preparation of homogenate, as explained previously. Relative organ weights were obtained using the formula [relative organ weight = (organ weight/body weight) × 100].

Histopathology. Paraffin-embedded tissue samples from bursa, proventriculus, spleen, and thymus from each bird were sectioned, mounted, stained using hematoxylin and eosin (HE), and examined in a blinded fashion (as to treatment for lesions) using light microscopy. All sections of bursa and proventriculus were assigned a lesion severity score. A lesion score of 1 represented no lesions. For bursal sections, a score of 2 was defined as mild variation in follicle size, 3 as moderate variation in follicle size, and 4 as either necrosis or follicle atrophy. For proventricular sections, a score of 2 was defined as mild glandular lumenal ectasia, 3 as ectasia plus lymphoid infiltrates in the interglandular interstitium, and 4 as either acute glandular necrosis or severe fibrosis with lymphoid infiltrates. Also, spleen and thymus were examined for the presence of lesions.

Serology. Serum samples obtained at 14 and 21 days of age were examined for antibody to IBDV, IBV, NDV, CAV, and reovirus using commercially available ELISA tests (IDEXX Laboratories, Inc.).

Real-time RT-PCR. RNA was extracted from formalin-fixed, paraffin-embedded bursas and proventriculi and examined for IBDV nucleic acid by real-time RT-PCR (25). Sections totaling 50 μm in thickness were cut from each formalin-fixed, paraffin-embedded tissue block, deparaffinized in HemoDe (Fisher Scientific, Pittsburgh, PA), washed with 100% ethanol, and digested with 25 μg/ml proteinase K (Sigma Chemical Co.) for 1 hr at 50 C. RNA was extracted using Trizol (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer's recommendations, diluted in 25 μl of 90% dimethyl sulfoxide, and frozen at −80 C until assayed. Extracted RNA was denatured at 95 C for 5 min and put on ice.

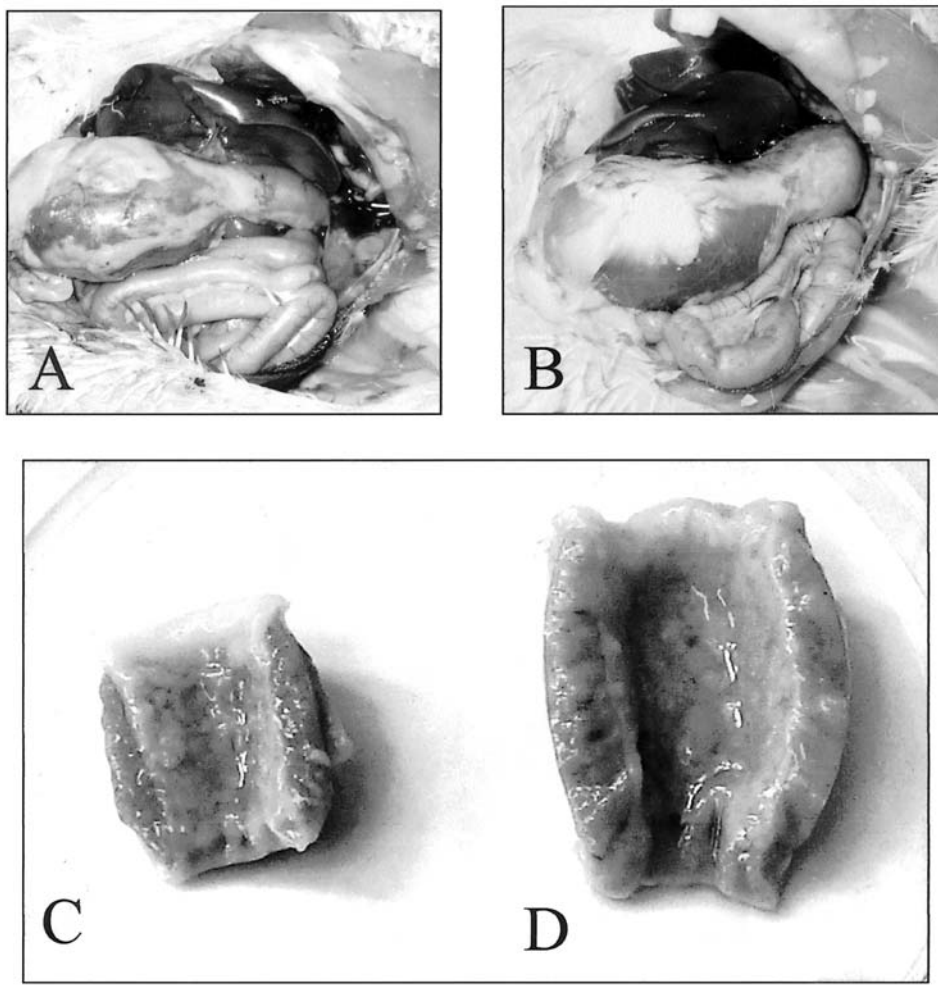

Fig. 1. Photographs of proventriculi from broiler chickens (14 days of age) inoculated with saline (A and C) or with infectious proventricular homogenate (B and D). Increase in size of the proventriculus and gastric isthmus and a white lobular pattern in a thickened mucosa can be observed in chickens with induced proventriculitis.

An RT-PCR was performed using reagents from the Light Cycler-RNA Amplification SYBR® Green I Kit (ROCHE Molecular Biochemicals, Indianapolis, IN). The primers used were designed to amplify a 400-base pair (bp) segment of the IBDV genome shared by all strains, which flanks a hypervariable region of the VP2 gene. Primer sequences were B4 5′ TCTTGGGTATGTGAGGCTTG and B4 3′ GGATGTGATTGGCTGGGTTA. Amplification and detection of specific products was also performed using a Light Cycler (ROCHE Molecular Biochemicals) according to the manufacturer's recommendations. Briefly, RT was done at 55 C for 10 min, followed by denaturation at 95 C for 30 sec. Forty PCR cycles were performed, consisting of denaturation (95 C for 1 sec), hybridization (55 C for 10 sec), and extension (72 C for 13 sec). A melting curve analysis was done with an initial denaturation at 95 C. DNA melting was accomplished with an initial temperature of 65 C for 10 sec and a gradual temperature increase of 0.1 C/sec until 95 C was reached. The melting temperature of the expected 400-bp amplicon was between 82 C and 84 C. This estimated melting temperature was used to confirm the identity of IBDV-specific products obtained using real-time RT-PCR. Additional confirmation of specific amplification was done using routine gel electrophoretic techniques of the PCR products on 2% agarose (Sigma Chemical Co.), followed by ethidium bromide staining.

Statistical analysis. The body weight gain, relative bursal and proventricular weights, and bursal and proventricular lesion scores were analyzed using

Table 2. Body weight gain (g) of commercial broilers (trials 1 and 2) and SPF broilers (trial 3) orally challenged at 7 days of age with sterile saline, negative proventricular homogenate (–PV), or positive proventricular homogenate (+PV) and necropsied at 7, 14, and 21 days postinoculation (dpi) (mean ± standard deviation).[A]

Groups	Trial 1	Trial 2	Trial 3
Day 14 (7 dpi)			
1. Saline	360.5 ± 34.3[a]	403.5 ± 14.5[a]	146.2 ± 10.2[a]
2. –PV	392.0 ± 7.16[a]	411.2 ± 31.8[a]	160.0 ± 16.7[a]
3. +PV	349.0 ± 24.9[a]	356.6 ± 45.0[ab]	147.5 ± 10.1[a]
4. IBDV	406.0 ± 42.6[a]	400.8 ± 26.1[a]	131.2 ± 13.2[ab]
5. CP	329.2 ± 95.9[a]	326.6 ± 50.8[ab]	76.7 ± 21.6[c]
6. CS	332.0 ± 83.7[a]	360.7 ± 54.7[ab]	128.7 ± 7.5[ab]
7. IBDV/+PV	340.0 ± 25.9[a]	361.8 ± 28.4[ab]	130.7 ± 2.7[ab]
8. CP/+PV	174.7 ± 40.5[b]	220.1 ± 43.0[b]	84.7 ± 8.1[c]
9. CS/+PV	370.7 ± 29.9[a]	290.3 ± 76.2[ab]	114.7 ± 8.13[b]
Day 21 (14 dpi)			
1. Saline	800.2 ± 26.1[a]	807.7 ± 39.1[a]	258.7 ± 18.7[ab]
2. –PV	831.7 ± 67.5[a]	714.4 ± 52.5[a]	294.0 ± 19.3[a]
3. +PV	807.7 ± 45.9[a]	689.0 ± 24.3[ab]	285.2 ± 24.2[a]
4. IBDV	741.2 ± 104.5[a]	773.4 ± 8.1[a]	254.2 ± 32.8[ab]
5. CP	733.2 ± 65.8[a]	549.0 ± 80.0[ab]	144.2 ± 39.1[c]
6. CS	816.2 ± 43.8[a]	506.2 ± 75.8[ab]	229.5 ± 256[ab]
7. IBDV/+PV	729.2 ± 123.9[a]	712.5 ± 81.9[ab]	249.5 ± 43.2[ab]
8. CP/+PV	539.2 ± 77.5[b]	392.2 ± 148.7[b]	169.5 ± 36.5[c]
9. CS/+PV	658.0 ± 72.0[ab]	528.4 ± 157.6[ab]	213.5 ± 11.3[b]
Day 28 (21 dpi)			
1. Saline			561.3 ± 73.0[a]
2. –PV			561.0 ± 109.9[a]
3. +PV			532.0 ± 97.5[a]
4. IBDV			518.6 ± 92.6[ab]
5. CP			316.0 ± 67.2[b]
6. CS			484.0 ± 68.9[ab]
7. IBDV/+PV			553.0 ± 92.9[a]
8. CP/+PV			393.0 ± 95.3[ab]
9. CS/+PV			422.0 ± 74.1[ab]

[A]Means within a column and time point with no common lowercase superscript are significantly different ($P < 0.05$). Means calculated from four birds in each group.

analysis of variance and means comparisons for all pairs using Tukey–Kramer HSD. Significance was assumed at the 0.05 level of probability.

RESULTS

Control groups. *Proventricular homogenate controls.* Chickens inoculated only with saline or negative proventricular homogenate (–PV) did not develop proventriculitis in any of the three trials. Macroscopic lesions were not observed when examined at necropsy (Fig. 1). Mean body weight gain and relative proventriculus weight for these two groups were very similar (Tables 2 and 3, respectively). Mild microscopic lesions consisting mainly of mild lumenal ectasia of the proventricular glands were present in some of these birds (Table 4). Chickens that were inoculated only with positive proventricular homogenate (+PV) had no significant suppression of weight gain compared to saline and –PV groups in all three trials (Table 2). There was a trend toward enlargement of the proventriculus in chickens that received the positive proventricular homogenate. Increased microscopic lesions were present in the proventriculus of chickens that received positive proventricular homogenate in trials 1 and 2 at 7 and 14 days postinoculation and in trial 3 at 14 days postinoculation (Table 4). At necropsy, the proventriculus of these chickens were enlarged and swollen, with plaques or mottling visible on the

Table 3. Relative proventriculus weight (% body weight) of commercial broilers (trials 1 and 2) and SPF broilers (trial 3) orally challenged at 7 days of age with sterile saline, negative proventricular homogenate (–PV), or positive proventricular homogenate (+PV) and necropsied at 7, 14, and 21 days postinoculation (dpi) (mean ± standard deviation).[A]

Groups	Trial 1	Trial 2	Trial 3
Day 14 (7 dpi)			
1. Saline	0.602 ± .051[a]	0.582 ± .047[a]	0.677 ± .097[a]
2. –PV	0.654 ± .042[ab]	0.562 ± .040[a]	0.707 ± .058[ab]
3. +PV	0.932 ± .023[ab]	0.812 ± .250[a]	0.925 ± .750[abc]
4. IBDV	0.550 ± .045[a]	0.670 ± .083[a]	0.685 ± .120[a]
5. CP	0.754 ± .098[ab]	0.685 ± .023[a]	0.965 ± .054[abc]
6. CS	0.670 ± .080[ab]	0.696 ± .064[a]	0.892 ± .180[abc]
7. IBDV/+PV	0.962 ± .220[b]	0.770 ± .153[a]	1.010 ± .212[c]
8. CP/+PV	1.406 ± .330[c]	1.002 ± .208[b]	0.985 ± .105[bc]
9. CS/+PV	0.930 ± .095[ab]	0.895 ± .175[a]	1.020 ± .099[c]
Day 21 (14 dpi)			
1. Saline	0.540 ± .075[a]	0.473 ± .030[a]	0.552 ± 061[a]
2. –PV	0.510 ± .060[a]	0.610 ± .140[a]	0.582 ± .022[a]
3. +PV	0.922 ± .194[ab]	0.743 ± .089[a]	0.745 ± .140[abcd]
4. IBDV	0.532 ± .072[a]	0.480 ± .036[a]	0.650 ± .083[abc]
5. CP	0.490 ± .057[a]	0.540 ± .045[a]	0.852 ± .140[bcd]
6. CS	0.535 ± .050[a]	0.580 ± .060[a]	0.685 ± .100[abc]
7. IBDV/+PV	0.900 ± .204[ab]	0.723 ± .130[a]	0.820 ± .110[abcd]
8. CP/+PV	0.950 ± .154[ab]	0.706 ± .210[a]	1.020 ± .152[d]
9. CS/+PV	1.202 ± .470[b]	0.886 ± .370[a]	0.927 ± .170[bcd]
Day 28 (21 dpi)			
1. Saline			0.463 ± .083[a]
2. –PV			0.436 ± .073[a]
3. +PV			0.580 ± .111[a]
4. IBDV			0.483 ± .149[a]
5. CP			0.676 ± .005[a]
6. CS			0.546 ± .096[a]
7. IBDV/+PV			0.506 ± .046[a]
8. CP/+PV			0.640 ± .103[a]
9. CS/+PV			0.970 ± .261[b]

[A]Means within a column and time point with no common lowercase superscript are significantly different ($P < 0.05$). Means calculated from four birds in each group.

serosal surface, dilation of the gastric isthmus, and mucosal lesions (flattened papillae, with secretion of white fluid) (Fig. 1). Microscopically, at 7 days postinoculation, acute necrosis of the glandular epithelium was present. Collecting sinuses of the glands were dilated and contained desquamated epithelium. Nuclei of the glandular epithelial cells were enlarged and pale, with marginated chromatin. Lymphocytic infiltrates were present as sheets in the lamina propria of the mucosa and expanded the glandular epithelium between the epithelium of the ducts and the glands (Fig. 2). At 14 days postinoculation, glandular epithelium was replaced by ductal epithelium. Lymphocyte infiltrates and germinal center formation were present in the glands and mucosa (Fig. 2). In trial 3, chickens that were inoculated with +PV showed similar mild to moderate lesions in the proventriculus at 21 days postinoculation but no significant increase in the size of the proventriculus compared to saline or –PV controls. Small germinal centers were present in the glands (Fig. 2) of +PV-dosed chickens but not in those of chickens given saline or –PV.

No lesions or differences in relative organ weight of the bursa were observed between chickens that received saline, –PV, or +PV (Tables 5, 6).

Immunosuppression controls. Commercial broilers (group 4) in trials 1 and 2, treated with IBDV strains Variant E and STC, respectively, had no signs of IBDV infection at 7 and 14 days postinoculation.

Table 4. Incidence and scoring of the severity of proventricular lesions in commercial broilers (trials 1 and 2) and SPF broilers (trial 3) orally challenged at 7 days of age with sterile saline, negative proventricular homogenate (–PV), or positive proventricular homogenate (+PV) and necropsied at 7, 14, and 21 days postinoculation (dpi).[A]

Groups	Trial 1		Trial 2		Trial 3	
Day 14 (7 dpi)						
1. Saline	1.00[aB]	0/4[C]	1.00[a]	0/4	1.50[a]	2/4
2. −PV	1.00[a]	0/4	1.00[a]	0/4	1.75[a]	2/4
3. +PV	3.00[b]	3/4	2.50[b]	2/4	2.50[ab]	3/4
4. IBDV	1.00[a]	0/4	1.25[a]	1/4	1.50[a]	2/4
5. CP	1.25[a]	1/4	1.25[a]	1/4	1.00[a]	0/4
6. CS	2.00[a]	2/4	1.00[a]	0/4	1.50[a]	2/4
7. IBDV/+PV	3.00[b]	3/4	2.00[ab]	2/4	2.50[ab]	3/4
8. CP/+PV	2.50[ab]	3/4	2.50[b]	3/4	1.25[a]	1/4
9. CS/+PV	3.50[b]	4/4	2.75[b]	3/4	3.25[b]	4/4
Day 21 (14 dpi)						
1. Saline	1.25[a]	1/4	1.00[a]	0/4	1.25[a]	2/4
2. −PV	1.00[a]	0/4	1.50[a]	2/4	1.50[a]	2/4
3. +PV	3.75[b]	4/4	3.50[b]	4/4	3.25[b]	4/4
4. IBDV	1.50[a]	2/4	1.25[a]	1/4	1.00[a]	0/4
5. CP	1.25[a]	1/4	1.25[a]	1/4	1.25[a]	1/4
6. CS	1.25[a]	1/4	1.00[a]	0/4	1.50[a]	2/4
7. IBDV/+PV	3.25[b]	4/4	3.50[b]	4/4	2.50[ab]	2/4
8. CP/+PV	3.00[b]	4/4	2.50[ab]	3/4	2.75[ab]	3/4
9. CS/+PV	4.00[b]	4/4	4.00[b]	4/4	3.25[b]	4/4
Day 28 (21 dpi)						
1. Saline					1.25[a]	1/4
2. −PV					1.50[a]	2/4
3. +PV					1.50[a]	2/4
4. IBDV					1.25[a]	1/4
5. CP					1.25[a]	1/4
6. CS					1.50[a]	2/4
7. IBDV/+PV					1.50[a]	2/4
8. CP/+PV					1.50[a]	2/4
9. CS/+PV					3.50[b]	4/4

[A]Means within a column and trial with no common lowercase superscript are significantly different ($P < 0.05$). Means calculated from four birds in each group.

[B]Proventriculus score: 1: no lesions; 2: mild glandular lumenal ectasia; 3: ectasia plus lymphoid infiltrates in the interglandular interstitium; and 4: either acute glandular necrosis or severe fibrosis with lymphoid infiltrates.

[C]Number of birds with mild, moderate, or severe lesions in the proventriculus/number of birds necropsied.

Their bursas had no significant microscopic lesions, no difference in relative organ weight when compared to controls (Tables 5, 6), and were negative for IBDV by RT-PCR. CBH response and humoral response to NDV vaccination was similar to the saline control group (Figs. 4, 5), all of which indicates that challenge with IBDV in these birds did not produce IBDV infection. However, SPF broiler chickens in trial 3 exposed to IBDV strain STC did have signs of depression at 7 days postinoculation, and their bursas were significantly smaller than those of saline control chickens at 7, 14, and 21 days postinoculation (Table 5). Severe microscopic lesions were also observed (Table 6), and bursas were positive for IBDV by RT-PCR. Humoral immune response to NDV vaccination was significantly lower than that observed in saline controls (Fig. 5).

CP control chickens (group 5) in all three trials tended to be smaller than chickens from the other groups as a result of a reduction in their weight gain. This reduction was significant in the SPF broilers in trial 3 (Table 2). These chickens also had decreased feathering and appeared weak. The bursas of these chickens were significantly smaller in all three trials (Table 5), and marked lymphocytic depletion and atrophy of the bursa were observed (Table 6). A small reduction of CBH response was observed in these birds (Fig. 4), and humoral response to NDV vaccination was significantly reduced (Fig. 5).

CS control chickens (group 6) in trials 1 and 2 appeared normal (similar to saline controls). Although their weight gain was reduced, it was not significantly different from that of the saline controls (Table 2). Weight gain in chickens in trial 3 was reduced at 7 and 21 days postinoculation. Bursas of birds treated with CS had no lesions, and there was no difference in size of bursas compared to saline controls (Tables 5, 6). Thymuses did not have any significant lesions, but the CMI immune capacity was significantly reduced (Fig. 4). The CBH-1 and CBH-2 responses were decreased ($P < 0.05$) compared to those of the saline control group. The humoral immune response, measured by antibody production after NDV vaccination, was not affected (Fig. 5).

The effect of the immunosuppressive treatments (IBDV, CP, and CS) on the proventriculus relative weight or presence of lesions was very mild and was not significantly different than that observed in saline or –PV controls (Tables 3, 4).

Experimental groups. *Body weight gain.* Chickens treated with CP and +PV had a significant reduction in body weight gain compared to the control groups (saline, –PV, and +PV), including those given CP only, in trials 1 at 7 and 14 days postinoculation and trial 2 at 7 days postinoculation (Table 2). The combination of CS

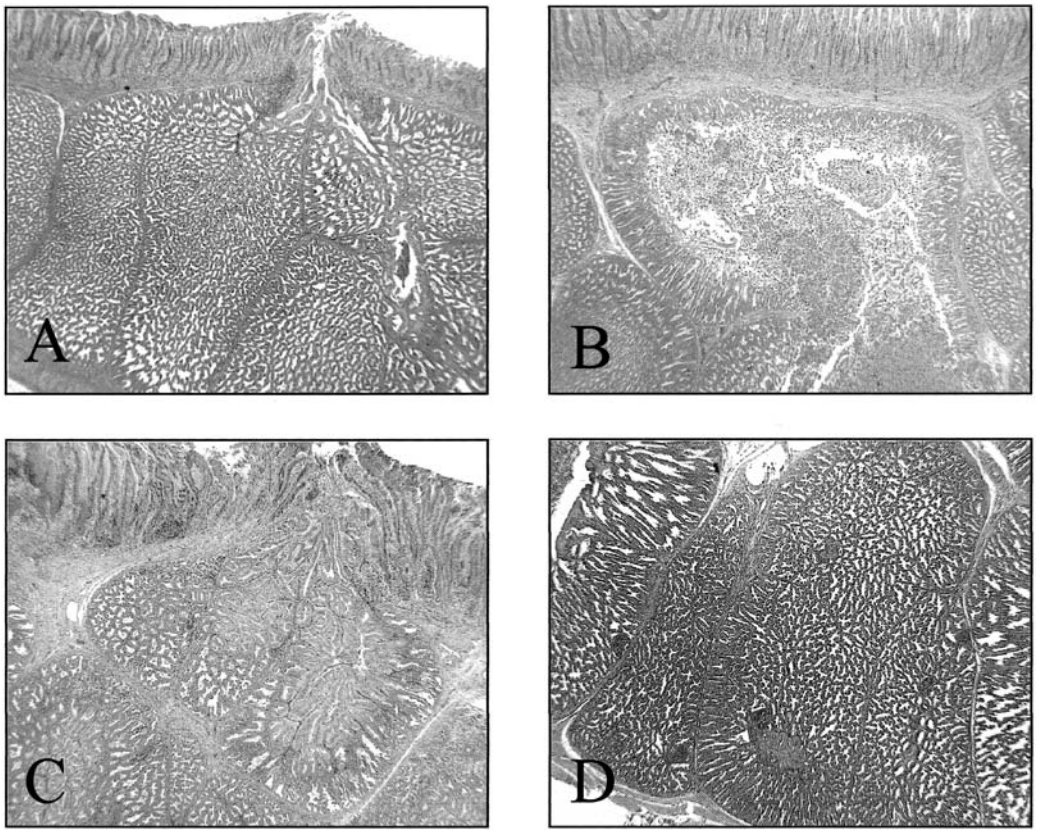

Fig. 2. Photomicrographs of proventriculi: (A) normal proventriculus of chickens inoculated with saline (negative control) (7 days postinoculation), (B) proventriculitis in chickens inoculated with positive proventricular homogenate (+PV) (7 days postinoculation) with necrosis of the glandular epithelium, coalescing of glands, and diffuse lymphocytic infiltration in glands and mucosa; (C) proventriculitis in chickens inoculated with +PV (14 days postinoculation), with ductal epithelium replacing glandular epithelium; (D) proventriculus in SPF broilers inoculated with +PV (21 days postinoculation), with small germinal centers. HE, 10×.

and +PV had a detrimental effect on weight gain in trial 2 at 14 days postinoculation and trial 3 at 7 and 21 days postinoculation, but the difference from chickens given CS only was not significant in any instance.

Organ relative weights and lesions. No significant difference was observed between control and experimental groups for spleen and thymus in any of the trials (data not shown). The exceptions were the chickens treated with CP, in which, at 7 days postinoculation, thymuses were smaller than those of the rest of the groups, but by 14 days postinoculation they were the same as controls. In all three trials, birds treated with CP and +PV had a significant decrease in bursal size and developed high lesion scores, but these results were no different than those noted in CP controls (Tables 5, 6). In trial 3, lesions and a significant decrease in size of the bursa occurred in chickens that were challenged with IBDV and exposed to +PV, similar to that observed in the IBDV controls (Tables 5, 6). These bursas were also IBDV positive by RT-PCR.

Relative proventricular weight of chickens that were immunosuppressed and treated with +PV was increased at 7 and 14 days postinoculation when compared to that of the control chickens (saline and −PV), but in most cases there was no significant difference when compared to the +PV controls. Chickens in trial 1 and 2 (at 7 days postinoculation) treated with the combination of CP/+PV had a significant increase in relative proventricular weight relative to the +PV controls (Table 3). The lesion score of the proventriculi from all immunosuppressed birds treated with +PV

Table 5. Bursa relative weights (% body weight) of commercial broilers (trials 1 and 2) and SPF broilers (trial 3) orally challenged at 7 days of age with sterile saline, negative proventricular homogenate (−PV), or positive proventricular homogenate (+PV) and necropsied at 7, 14, and 21 days postinoculation (dpi) (mean ± standard deviation).[A]

Groups	Trial 1	Trial 2	Trial 3
Day 14 (7 dpi)			
1. Saline	0.127 ± .075[a]	0.220 ± .034[a]	0.310 ± .045[a]
2. −PV	0.170 ± .027[a]	0.160 ± .039[a]	0.320 ± .029[a]
3. +PV	0.195 ± .020[a]	0.205 ± .035[a]	0.365 ± .090[a]
4. IBDV	0.160 ± .010[a]	0.165 ± .052[a]	0.065 ± .010[b]
5. CP	0.032 ± .009[b]	0.060 ± .008[b]	0.017 ± .015[b]
6. CS	0.150 ± .017[a]	0.213 ± .020[a]	0.370 ± .067[a]
7. IBDV/+PV	0.200 ± .026[a]	0.152 ± .035[a]	0.172 ± .009[b]
8. CP/+PV	0.055 ± .012[b]	0.060 ± .024[b]	0.085 ± .005[b]
9. CS/+PV	0.202 ± .022[a]	0.182 ± .088[a]	0.325 ± .098[a]
Day 21 (14 dpi)			
1. Saline	0.250 ± .098[a]	0.230 ± .081[a]	0.340 ± .065[a]
2. −PV	0.272 ± .090[a]	0.230 ± .095[a]	0.304 ± .045[a]
3. +PV	0.225 ± .036[a]	0.196 ± .075[a]	0.305 ± .084[a]
4. IBDV	0.190 ± .060[a]	0.193 ± .068[a]	0.112 ± .009[b]
5. CP	0.032 ± .009[b]	0.050 ± .017[b]	0.055 ± .012[b]
6. CS	0.232 ± .033[a]	0.153 ± .055[a]	0.387 ± .098[a]
7. IBDV/+PV	0.225 ± .042[a]	0.193 ± .064[a]	0.080 ± .049[b]
8. CP/+PV	0.070 ± .018[b]	0.063 ± .020[b]	0.070 ± .040[b]
9. CS/+PV	0.282 ± .052[a]	0.190 ± .066[a]	0.377 ± .054[a]
Day 28 (21 dpi)			
1. Saline			0.233 ± .023[a]
2. −PV			0.243 ± .015[a]
3. +PV			0.316 ± .047[a]
4. IBDV			0.073 ± .036[b]
5. CP			0.066 ± .005[b]
6. CS			0.325 ± .051[a]
7. IBDV/+PV			0.060 ± .010[b]
8. CP/+PV			0.060 ± .036[b]
9. CS/+PV			0.463 ± .027[a]

[A]Means within a column and time point with no common lowercase superscript are significantly different ($P < 0.05$). Means calculated from four birds in each group.

was also similar to those observed in the +PV control groups at 7 and 14 days postinoculation (Table 4), although there was an increase in the incidence of proventriculitis and a difference in the appearance and severity of the lesions observed in the birds treated with CS. This was more evident in the SPF broilers, in which all birds treated with the combination of CS and +PV had moderate to severe proventriculitis. CS/+PV scores were significantly higher than all other treatments at 21 days postinoculation in trial 3. In all three trials, the incidence and severity of proventriculitis was higher at 14 days postinoculation than 7 days postinoculation. In trial 3 at 21 days postinoculation, the relative weight and lesion score of the proventriculi of all birds that received +PV was similar to that of the negative controls, with the exception of the chickens treated with CP/+PV, in which scoring and weight remained significantly higher than birds in the other groups (Tables 3, 4).

Chickens treated with +PV in all three trials, regardless of the immunosuppression treatment utilized, had acute necrosis of the proventricular glands at 7 days postinoculation, with some lymphocyte infiltrates, mostly in the mucosa. In some cases, lymphocyte infiltrates also were present in the glands in the form of sheets. Hemorrhage and congestion were also sometimes present. Birds

treated with CS had more severe lesions, with destruction and coalescence of the glands.

At 14 days postinoculation, chickens treated with IBDV and +PV or with CP and +PV had metaplastic replacement of proventricular glandular secretory epithelium by ductal epithelium and lymphocyte infiltrates, as observed in the +PV only–treated chickens. Proventricular lymphoid germinal centers were smaller, or not present, in birds treated with CP (in all three trials) or IBDV (in trial 3). Chickens treated with CS and +PV in trials 1 and 2 still had acute necrosis at 14 days postinoculation, reduced lymphocyte infiltration and variable germinal center formation, and minimal metaplasia (Fig. 3).

At 21 days postinoculation, SPF broilers treated with IBD and +PV or with CP and +PV had mild to moderate lesions, with very little lymphocyte infiltration. These were mostly in the form of small germinal centers. Chickens treated with CS and +PV had severe lesions consisting of acute necrosis of the glandular epithelium, coalescing of glands, and small and multiple germinal centers.

Serology. Chickens from all groups in trial 1 had ELISA titers against IBDV, IBV, and CAV at 14 days of age (7 days postinoculation) and had no titers against NDV and reovirus. These IBDV, IBV, and CAV titers decreased but were still present at 21 days of age (14 days postinoculation). Chickens in trial 2 had titers for IBDV, IBV, NDV, and CAV at 14 days of age (7 days postinoculation), but no titers for reovirus. These titers decreased at 21 days of age (14 days postinoculation). In both trials, some of the chickens that received –PV or +PV (with the exception of birds treated with CP) developed titers against reovirus at 21 days of age (14 days postinoculation).

SPF broiler chickens (trial 3) at 14 days of age (7 days postinoculation) were seronegative for NDV, IBV, reovirus, and CAV. They also were negative for IBDV, with the exception of those challenged with IBDV, which developed bursal disease and had seroconversion at 14, 21, and 30 days of age (7, 14, and 21 days postinoculation). At 21 and 30 days of age (14 and 21 days postinoculation), some of the birds that received +PV, but that were not treated with CP, had titers against IBV, NDV, and reovirus. Birds that received –PV did not have titers for IBDV, IBV, or NDV, but some did have titers for reovirus. All birds were negative for CAV at all time points.

IBDV RT-PCR. IBDV was not detected in paraffin-embedded bursas or proventriculi from any of the birds in trials 1 or 2. In trial 3, IBDV was detected at 7, 14, and 21 days postinoculation in paraffin-embedded bursas from all IBDV-challenged birds. It was not detected in any of the proventriculi from these birds or in bursas or proventriculi from chickens in the other groups in trial 3.

Table 6. Incidence and scoring of the severity in bursal lesions of commercial broilers (trials 1 and 2) and SPF broilers (trial 3) orally challenged at 7 days of age with sterile saline, negative proventricular homogenate (–PV), or positive proventricular homogenate (+PV) and necropsied at 7, 14, and 21 days postinoculation (dpi).[A]

Groups	Trial 1		Trial 2		Trial 3	
Day 14 (7 dpi)						
1. Saline	1.00[aB]	0/4[C]	1.50[a]	2/4	1.25[a]	1/4
2. –PV	1.00[a]	0/4	2.00[a]	4/4	1.25[a]	1/4
3. +PV	1.75[a]	2/4	2.25[a]	4/4	2.50[b]	4/4
4. IBDV	1.25[a]	1/4	1.75[a]	3/4	4.00[c]	4/4
5. CP	4.00[b]	4/4	4.00[b]	4/4	4.00[c]	4/4
6. CS	1.75[a]	3/4	1.25[a]	1/4	1.75[ab]	3/4
7. IBDV/+PV	1.75[a]	3/4	1.25[a]	1/4	4.00[c]	4/4
8. CP/+PV	4.00[b]	4/4	4.00[b]	4/4	4.00[c]	4/4
9. CS/+PV	1.50[a]	2/4	1.50[a]	3/4	2.00[ab]	4/4
Day 21 (14 dpi)						
1. Saline	1.25[a]	1/4	1.25[a]	1/4	1.25[a]	1/4
2. –PV	1.25[a]	1/4	1.00[a]	0/4	1.50[a]	2/4
3. +PV	1.25[a]	1/4	1.25[a]	1/4	1.75[a]	2/4
4. IBDV	1.00[a]	0/4	1.25[a]	1/4	4.00[b]	4/4
5. CP	4.00[b]	4/4	4.00[b]	4/4	4.00[b]	4/4
6. CS	1.50[a]	2/4	1.25[a]	1/4	1.50[a]	2/4
7. IBDV/+PV	1.00[a]	0/4	2.00[a]	4/4	4.00[b]	4/4
8. CP/+PV	4.00[b]	4/4	4.00[b]	4/4	4.00[b]	4/4
9. CS/+PV	1.75[a]	2/4	1.00[a]	0/4	1.00[a]	0/4
Day 28 (21 dpi)						
1. Saline					1.00[a]	0/4
2. –PV					1.25[a]	1/4
3. +PV					1.00[a]	0/4
4. IBDV					4.00[b]	4/4
5. CP					4.00[b]	4/4
6. CS					1.00[a]	0/4
7. IBDV/+PV					4.00[b]	4/4
8. CP/+PV					4.00[b]	4/4
9. CS/+PV					1.00[a]	0/4

[A]Means within a column and trial with no common lowercase superscript are significantly different ($P < 0.05$). Means calculated from four birds in each group.

[B]Bursa score: 1: no lesions; 2: mild variation in follicle size; 3: moderate variation in size of follicles; and 4: either necrosis or follicle atrophy.

[C]Number of birds with mild, moderate, or severe lesions in the bursa/number of birds necropsied.

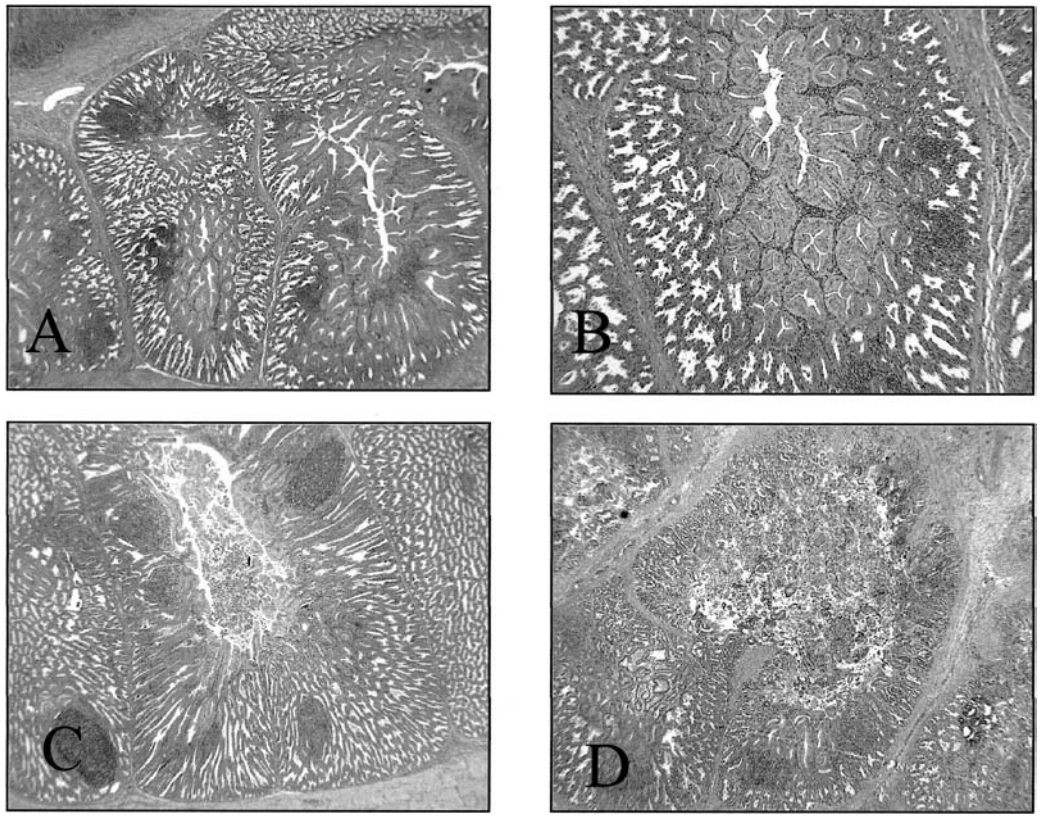

Fig. 3. Photomicrographs of proventriculi from broiler chickens inoculated with positive proventricular homogenate (+PV) (14 days postinoculation). (A and B) Treated with CP and +PV, with metaplastic replacement of proventricular glandular epithelium by ductal epithelium with minimal necrosis. (C and D) Treated with CS and +PV, with acute necrosis of the epithelium with coalescing glands and variable germinal center formation. HE, 10× and 25×.

DISCUSSION

The relationship between IBDV and proventriculitis is not clear. Both gross and microscopic lesions of the proventriculus have been produced by IBDV challenge in leghorn chickens (24), and vaccination against IBDV has been reported to decrease the incidence of proventriculitis (7,15). However, proventriculitis was not produced by inoculation of SPF broilers with different strains of IBDV (25). Commercial chickens get exposed to IBDV early in life, and although mortality in unprotected flocks can be quite significant, the major concern for the poultry industry is IBDV's ability to cause immunosuppression. Immunosuppressed birds often fail to respond adequately to vaccination and are susceptible to secondary infections. The mechanisms of IBDV-induced immunosuppression are not fully understood. Both humoral and cellular immune responses are compromised (35). Inhibition of humoral immunity is more severe and is attributed to the destruction of immunoglobulin-producing B cells by the virus. IBDV-exposed chickens produce suboptimal levels of antibodies against a number of infectious and noninfectious antigens (35). Although T cells do not serve as targets for IBDV replication, cell-mediated immune responses of virus-exposed chickens seem to be compromised (5,18,33,35).

In our study, commercial broiler chickens (trials 1 and 2) inoculated with an infecting dose of IBDV did not develop disease, most likely because of the presence of maternal antibodies. No lesions were observed in their bursas, and RT-PCR did not detect any virus. Consequently, these birds were not immunosuppressed by IBDV as intended, and

they had a normal response to NDV vaccination. On the other hand, SPF broiler chickens were successfully infected with IBDV when intentionally challenged at 1 day of age. Their bursas were significantly smaller than those of controls, had lesions typical of IBDV infection, and were positive for the virus by RT-PCR. They also developed antibodies against IBDV and were immunosuppressed, as measured by their low seroconversion to NDV. However, infection with this particular strain of IBDV (STC) produced no proventriculitis.

CP treatment has been used to inhibit humoral immunity in order to determine its role in the pathogenesis of infectious pathogens of chickens (1,31). Chickens treated with CP had bursas that were significantly smaller and depleted of lymphocytes, and they did not develop specific antibody after NDV vaccination, demonstrating their humoral immunosuppression. Both CP and IBDV have minor effects on CMI (34,35). There was a mild depression of the CBH response in birds treated with IBDV (trial 3) or CP, but this was not significant when compared to that of controls.

Chickens from all three trials treated with CS exhibited a significantly decreased CBH response (6). CS prevents cytokine synthesis in T cells by blocking a later stage of T cell receptor–initiated signaling, reducing production of interleukin-2, and hence T cell proliferation (11,12,28). Humoral immune response of birds treated with CS was not significantly affected, and they developed anti-NDV antibodies following NDV vaccination.

The homogenate used to induce proventriculitis in trial 1 was known to contain IBDV (13). In an attempt to reproduce a proventriculitis as close to that observed in naturally occurring cases, commercial broilers with maternal antibodies to IBDV were used in trials 1 and 2. Inoculation of these chickens in trial 1 with the IBDV-bearing homogenate produced proventriculitis but no IBDV infection, since their anti-IBDV antibody was protective. Since proventriculitis still occurred, this indicates that proventriculitis was not directly produced by infection with the IBDV present in that homogenate, but this does not exclude IBDV as a potential contributing factor. In trials 2 and 3, proventriculitis was produced by inoculating birds with positive proventricular homogenate produced from birds with proventriculitis in trial 1. Excluding those challenged with IBDV intentionally, chickens given this homogenate in trials 2 and 3 developed proventriculitis but no IBDV infection. These data

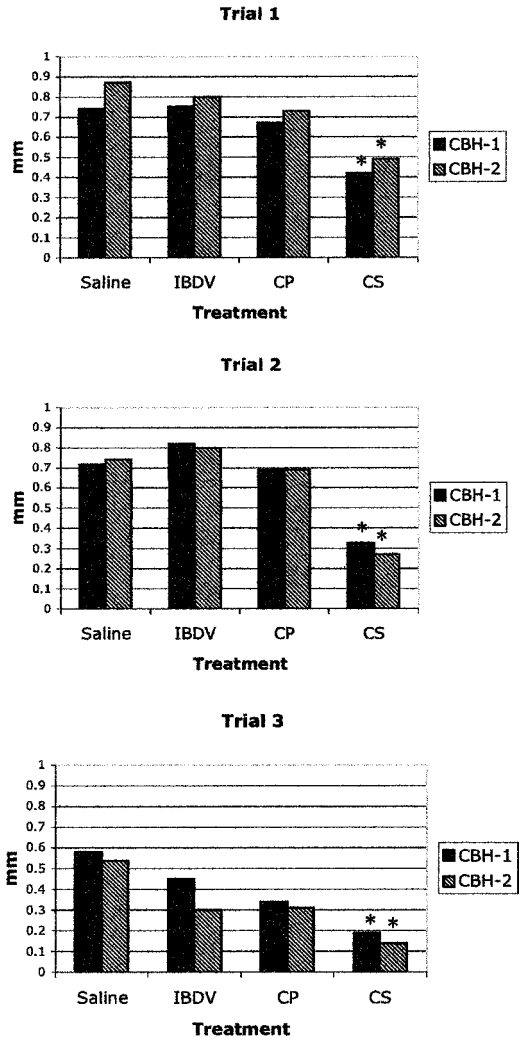

Fig. 4. Effect of immunosuppression treatments[A] on the cutaneous basophil hypersensitivity response[B] (CBH-1[C] and CBH-2[D]) induced by injection of phytohemagglutinin P (PHA-P) and physiological saline solution (PSS) in 14-day-old chickens from control groups. [A]IBDV treatment: 10^3 CID_{50} *per os* strains Variant E (trial 1) or STC (trials 2 and 3). CP treatment: 4 mg intraperitoneally for 4 days starting at 1 day of age. CS treatment: intramuscular injection of 50 mg/kg body weight every third day, starting at 1 day of age. [B]Data expressed as mean; $n = 4$. [C]CBH-1 = (skin thickness at 12 hr postinjection, left foot) − (preinjection skin thickness, left foot) (mm). [D]CBH-2 = (skin thickness, PHA-P injected foot) − (skin thickness, PSS-injected foot) (mm). Asterisks indicate values that are significantly different from saline group ($P < 0.05$).

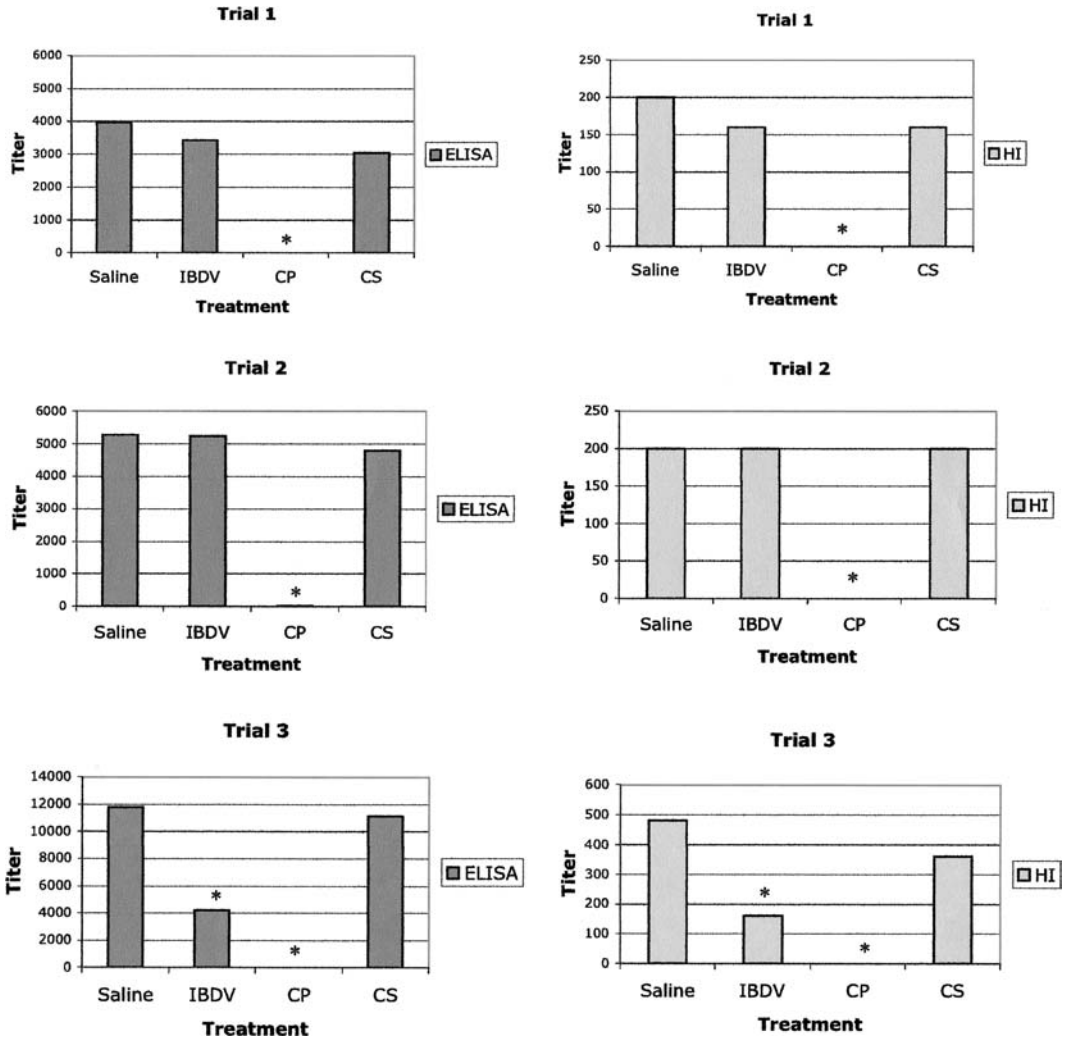

Fig. 5. Serologic responses to killed Newcastle disease (ND) vaccine in chickens inoculated with either sterile saline[A], infectious bursal disease virus[B] (IBDV), cyclophosphamide[C] (CP), or cyclosporine[D] (CS); 14 days postinoculation.[E] [A]Saline: 1 ml sterile saline *per os.* [B]IBDV treatment: 10^3 CID_{50} *per os* strains Variant E (trial 1) or STC (trials 2 and 3). [C]CP treatment: 4 mg intraperitoneal for 4 days starting at 1 day of age. [D]CS treatment: intramuscular injection of 50 mg/kg body weight every third day, starting at 1 day of age. [E]Mean titers for ELISA and hemagglutination inhibition calculated from four birds. Asterisks indicate values that are significantly different from saline group ($P < 0.05$).

indicate that our serial passage of the original proventricular homogenate through antibody-positive broiler chickens cleared it of IBDV and propagated the causative agent responsible for proventriculitis.

The proventriculitis produced in trial 1 was more severe than that produced in trials 2 and 3. This may be due to reduction in titer of the causative pathogen by *in vivo* passage in the presence of antibody. Even so, the incidences of proventriculitis within groups and the effects of immunosuppression on proventriculitis were similar across all three trials.

Immunosuppression induced by CP in all three trials, and by IBDV in trial 3, did not affect the incidence or lesion severity of the proventriculitis observed. Proventricular lesions observed in

chickens that received CP/+PV were similar to those observed in the +PV controls. There was acute glandular necrosis and some lymphocyte infiltrate at 7 days postinoculation, and glandular metaplasia with severe lymphocyte infiltrates at 14 days postinoculation. Both sheets and follicles of lymphocytes were present, representing T and B cells, respectively (22); however, in these birds, less follicle formation was observed. Although the lesions observed in the proventriculi of birds treated with CP/+PV were similar to those of the controls, at 7 days postinoculation, chickens from these groups in trials 1 and 2 had significantly higher proventriculus weights than did the +PV controls. This indicates a role of B cells in the early stages of proventriculitis, during which compromised production of antibodies could exacerbate the severity of the condition.

All chickens with T cell suppression due to CS and treated with +PV had equal or higher incidence and lesion scores of proventriculitis than did +PV controls. The proventricular relative weights also tended to be higher than those of +PV controls, being more evident in the SPF birds in trial 3, in which this difference was significant at 21 dpi. CMI responses have been suggested to play a key role in the elimination of avian enteric pathogens (1,20,36), and our data indicate that T cell functions play a role in controlling proventriculitis. The high incidence of lesions in the proventriculi of birds in trial 3 at 21 dpi, which were immunosuppressed with CP and treated with +PV, indicates the importance of T lymphocytes in the clearing and resolution of proventriculitis. It is well known that IBDV can affect the CMI response (5,18,33,35), and although we saw little effect of IBDV-induced immunosuppression on the severity of proventriculitis in this study, it is possible that preventing severe immunosuppression in the field through vaccination against IBDV could diminish the severity of proventriculitis.

Positive homogenates without other contaminating viruses are unavailable. Serologic results infer that the original positive proventricular homogenate used in this study contained IBV, NDV, and reovirus, because some dosed experimental chickens seroconverted to these agents. Passage of this homogenate in commercial broilers seemed to have eliminated IBDV, because SPFs challenged with the subsequent proventricular homogenate did not seroconvert to this virus or develop bursal disease. The objectives and experimental design of the present study were not designed to determine the role(s) of these other agents in proventriculitis, so no conclusions should be drawn from their presence here. Because not all birds that received +PV and developed proventriculitis seroconverted to these viruses, and since the birds that did seroconvert did not present more lesions in the proventriculus than the ones that didn't, it is most likely that infection with these viruses did not have an effect on the proventriculitis produced.

In conclusion, B cell immunosuppression, by either CP or IBDV, did not have an effect on the incidence of proventriculitis, and the lesions observed were similar to those produced by +PV alone. However, proventricular enlargement was more evident in these birds at 7 dpi, indicating that humoral response might be important in the early stages of the disease, probably via control of the causative agent by production of antibodies. T cell suppression by CS, on the other hand, did have an effect on the incidence of proventriculitis, and the lesions observed were more severe and lasted longer than in +PV controls.

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